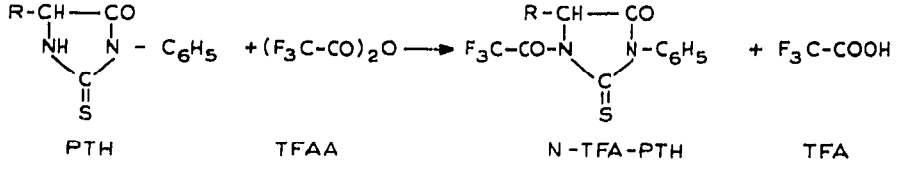
CHROM. 4500

Gas chromatographic analysis of amino acids as trifluoroacetylated phenylthiohydantoins*

The analysis of the 3-phenyl-2-thiohydantoin (PTH) derivatives of amino acids, used for the first time by EDMAN in the study of proteins[1,2], is commonly carried out by paper and thin-layer chromatography[3,4]. Only few significant papers on the gas chromatographic determination of the PTH derivatives of amino acids can be found in scientific literature[4]. It is as yet impossible to carry out a complete gas chromatographic examination of these compounds that would be very useful in the sequence analysis of peptides. The greatest difficulties arise from their insufficient volatility that makes it necessary to use high temperatures, glass columns, very small amounts of stationary phase, very long analysis times, etc.

We thought it possible to overcome in part these drawbacks by decreasing the polarity of the PTH's through their N-trifluoroacetylation:



Preparatoin of the N-trifluoroacetylated PTH's of amino acids

The PTH's of glycine, α-alanine, valine, leucine, isoleucine and proline were prepared by the EDMAN method[1]. The purity of the products obtained was checked spectrophotometrically according to the procedure described by SJÖQUIST[6]. To about

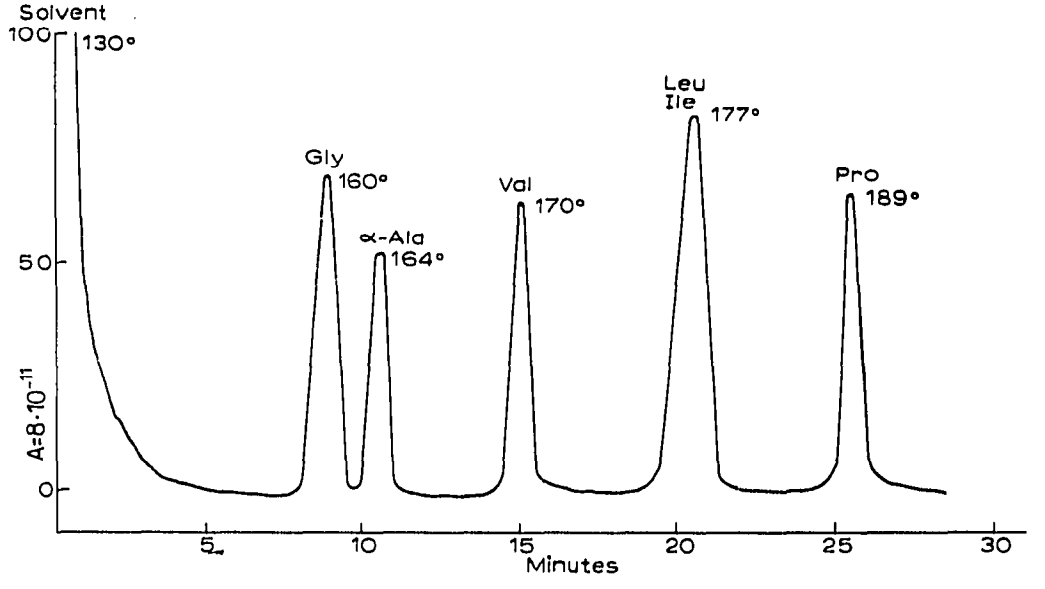


Fig. 1. Chromatogram obtained with the mixture of the six amino acids analysed.

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3 mg of each PTH 2 ml of methylene chloride and 0.3 ml of TFAA were added. The solution was kept at room temperature for 30 min and then analysed by gas chromatography.

Gas chromatographic procedure

A Varian-Aerograph 1520 B gas chromatograph with a flame ionisation detector was used. The analytical conditions were as follows: stainless-steel columns, 1.5 m by 3 mm I.D.; carrier gas, nitrogen, 20 ml/min; support, acid-washed, 68–80 mesh Chromosorb W; stationary phase, 5% S.E. 30; injector temperature, 220°; detector temperature, 250°; programmed oven temperature, initial value at 130°, from the initial value to 150° at 4°/min, from 150° to the end at 2°/min; amount of sample injected, 1 μl. An example of chromatogram is shown in Fig. 1.

The results of this preliminary research, carried out on the simplest amino acids, show that trifluoroacetylation allows the gas chromatographic analysis of their PTH derivatives (even with stainless-steel columns) at relatively low temperatures, even if a 5% concentration of the stationary phase is used. All the amino acids are completely separated except leucine and isoleucine which give one peak.

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